infoPoint

Detection of mycoplasma in cell culture

Introduction

Surveys of cultures from labs all over the world reveal a strong prevalence of contamination by mycoplasma and other mollicutes. Depending on the method of detection 10-40% of continuous cell lines have been tested positively. The species most frequently found are *Mycoplasma orale, M. fermentans* (human), *M. arginini, Acholeplasma laidlawii* (bovine), and *M. hominis* (swine).

Sources of contamination

There are various possible sources for contamination by mycoplasma. During recent years, a rising awareness of the problem may have changed the contribution of the individual sources. Culture reagents such as bovine serum have been a considerable source of contamination in the past. Today, most labs prefer mycoplasma-free tested sera.

Laboratory personnel may introduce mycoplasma into cultures, are now trained to avoid contamination during the handling of cultures. However, other sources are even more difficult to avoid. Any addition to the culture is relevant, such as virus suspensions, antibody solutions, or media ingredients. Mycoplasma from original tissue isolates contribute to less than 1% to the reported cases.

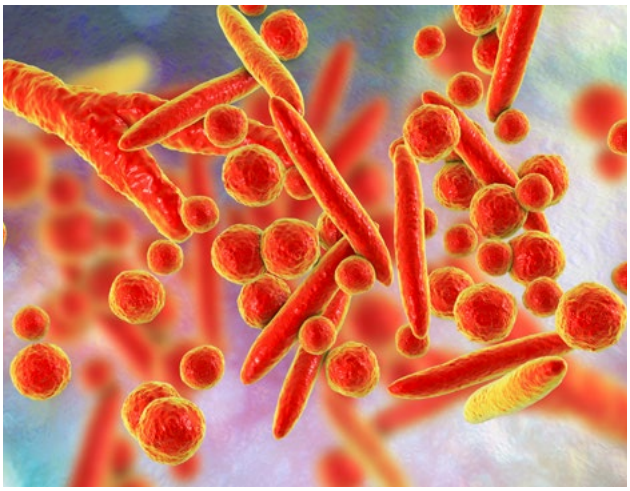
The most common source by far is cross-contamination from infected cultures. Labs exchange infected cultures and thereby inadvertently distribute mycoplasma.

PanReac AppliChem provides the tools for the detection of mycoplasmas for every cell culture laboratory.

For the detection by microscopy we are offering the proven fluorescent dye DAPI (product code A1001, available in pack sizes from 10 mg to 10 g).

Detection by PCR

In recent years the sensitive polymerase chain reaction (PCR) became a standard method for the detection of mycoplasma contamination in biological samples such as mammalian cell cultures. The PCR is established in almost all life science labs either as standard PCR or real time/quantitative PCR. For your preferred setup, we offer two different kits to choose from.



Keywords

- **Mycoplasma contamination**
- **Mycoplasma-induced cellular effects**
- **PCR detection of Mycoplasma**

The rRNA gene sequences of prokaryotes including mycoplasmas are well conserved, whereas the lengths and sequences of the spacer region in the rRNA differ from species to species.

The detection procedure utilizes the PCR for amplification of a conserved and mycoplasma-specific 16S rRNA gene region. This system does not allow the amplification of DNA originating from other sources, such as cultured cells or bacteria, which affect the detection result. Amplification of the gene sequence with PCR using this primer set enhances not only the sensitivity, but also the specificity of detection.

Amplified products are detected by agarose gel electrophoresis or by real time/quantitative PCR (qPCR Mycoplasma Test Kit, product code A9019).

PanReac AppliChem ITW Reagents

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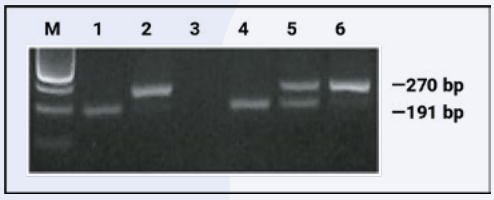
Mycoplasma detection by microscopy

Product number	Product name	CAS number	Pack sizes
A1001	DAPI BioChemica	28718-90-3	10 mg 100 mg 10 g

Mycoplasma detection kits using standard PCR

	Product code A8994 PCR Mycoplasma Test Kit II	Product code A9019 qPCR Mycoplasma Test Kit
Kit components	• Reaction Mix (including PCR primers, dNTPs) • Reaction Buffer Solution • PCR grade water • Positive template control • Internal control DNA Kit meets criteria of section 2.6.7 of Ph.Eur.	• Reaction Mix (including PCR primers, dNTPs) • Reaction Buffer Solution • PCR grade water • Positive template control • Internal control DNA
Taq DNA polymerase	not included	included
Form of delivery	single components, lyophilized	single components, lyophilized
Storage	2 - 8 °C	2 - 8 °C
Packaging	25 tests 50 tests 100 tests 	25 tests 



Possible PCR products of PCR Mycoplasma Test Kit II

1: Negative control
2: Positive control
3: Inhibited sample
4: Negative sample
5: Contaminated positive sample
6: Contaminated positive sample with high mycoplasma DNA concentration
M: DNA marker

IP-024EN

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